

PATENT CLAIMS

- 5 1. A method for the separation of cell fractions, comprising normal cells and altered cells, comprising the step of incubating the mixture of normal cells and altered cells in a hypotonic solution and the destruction of one or more cell fractions thereof.
- 10 2. A method according to claim 1, further comprising the subsequent step of collecting the non-destroyed cell fraction.
3. A method according to claim 1 or 2, further comprising the subsequent step of analysis of the cells of the collected cell fractions.
- 15 4. A method according to claim 3, wherein the analysis of the derived cells is through polymerase chain reaction (PCR).
5. A method according to any one of the preceding claims, wherein the mixture of normal cells and altered cells is derived from bodily fluids or tissue.
- 20 6. A method according to claim 5, wherein the bodily fluid is selected from the group comprising blood, urine, cerebrospinal fluid, bone marrow, lymph, ascites and sputum.
- 25 7. A method according to any one of the preceding claims, wherein the altered cells are tumor cells.
- 30 8. A method according to any one of the preceding claims, wherein the tumor cells are circulating and/or micrometastatic tumor cells.

9. A method according to any one of the preceding claims, wherein the normal cells are only mononuclear cells from the blood.

5 10. A method according to any one of the preceding claims, wherein the tumor cells are selected from the group consisting of the group of solid malignant tumors of epithelial origin (carcinomas).

10 11. A method according to any one of the preceding claims, wherein the osmolality of the hypotonic solution is below 100 mosm/kg.

15 12. A method according to any one of the preceding claims, wherein the hypotonic solution is a salt solution selected from the salts NaCl, KCl, NH₃Cl, Phosphate Buffered Saline (PBS), Hank's Balanced Salt Solution (HBBS) or mixtures thereof.

13. A method according to any one of the preceding claims, wherein the hypotonic solution further contains enzymes that degrade nucleic acid and/or protein-degrading enzymes.

20 14. A method according to any one of the preceding claims, wherein the hypotonic solution further contains RNase.

25 15. A method according to any one of claims 3 to 14, wherein the analysis of the derived cells comprises the determination of the expression of a tumor marker.

30 16. A method according to claim 15, wherein the tumor marker is selected from cytokeratin 18 (CK18), cytokeratin 19 (CK19), cytokeratin 20 (CK20) and further members of the cytokeratin family, carcinoembryonic antigen (CEA), ErbB2, ErbB3, epithelial mucin-1, epithelial mucin-18,

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guanylyl cyclase C, Cdx-1, Cdx-2, prostate specific antigen (PSA), prostate specific membrane antigen (PSMA), sucrose isomaltase, lactase, carbonic anhydrase, tyrosinase, thyroglobulin, tyrosine hydroxylase, neurone-specific glycoprotein, Desmoplakin I, epithelial glycoprotein 40 and gastrointestinal associated-associated antigen.

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17. Use of a method according to any one of claims 1 to 16 to detect the presence of altered cells, in particular tumor cells.

10 18. Use of a method according to any one of claims 1 to 16 for the diagnosis of metastatic cancer.

19. Kit to detect the presence of tumor cells in a sample, comprising

15 a) a hypotonic solution, and
b) primer to detect the presence of mRNA coding for a tumor marker.

20. Kit according to claim 19, further comprising

20 c) a RNA-stabilizing solution, comprising a highly-concentrated chaotropic salt.

21. Kit according to claim 19 or 20, wherein the hypotonic solution has an osmolality below 100 mosm/kg.

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22. Kit according to any one of the preceding claims, wherein the hypotonic solution is a salt solution, selected from the salts NaCl, KCl, NH₃Cl, Phosphate Buffered Saline (PBS), Hank's Balanced Salt Solution (HBBS) and mixtures thereof.

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23. Kit according to any one of the preceding claims for the diagnosis of metastatic cancer.

24. Kit according to any one of the preceding claims, wherein the marker is selected from the group comprising cytokeratin 18 (CK18), cytokeratin 19 (CK19), cytokeratin 20 (CK20) and further members of the cytokeratin family, carcinoembryonic antigen (CEA), ErbB2, ErbB3, epithelial mucin-1, epithelial mucin-18, guanylyl cyclase C, Cdx-1, Cdx-2, prostate specific antigen (PSA), prostate specific membrane antigen (PSMA), sucrose isomaltase, lactase, carbonic anhydrase, tyrosinase, thyroglobulin, tyrosine hydroxylase, neurone-specific glycoprotein, Desmoplakin I, epithelial glycoprotein 40 and gastrointestinal associated-associated antigen.

25. Use of a kit according to any one of claims 19 to 24 to detect the presence of tumor cells in a sample.